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Mouse Skin Tumorigenicity Studies of Indoor Coal and Wood Combustion Emissions from Homes of Residents in Xuan Wei, China with High Lung Cancer Mortality

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16. ABSTRACT
The rural Xuan Wei County, Yunnan Province, China, has an unusually high lung cancer mortality rate that cannot be attributed to tobacco smoke or occupational exposure. The lung cancer rate is associated with "smoky" coal, in contrast to wood or "smokeless" coal burned in unventilated homes. This study was conducted to characterize and compare mouse skin tumorigenicity of the coal and the wood combustion emissions and to link the resulting animal data to human lung cancer. Indoor air particles were collected from a central commune where the lung cancer mortality rate is high and smoky coal is the major fuel used, and also from a south western commune where lung cancer mortality rate is low and wood and smokeless coal are the major fuels used. The organic extracts of these indoor air particles were analyzed for polycyclic aromatic hydrocarbons (PAHs) and assayed for skin tumor initiation activity and complete carcinogenicity in SENCAR mice. Mouse skin was initiated with 1,2,5,10, and 20 mg of organic extracts of the emission particles during the first week, and one week after initiation the mice were promoted with 12-*O*-tetradecanoylphorbol-13-acetate [TPA, 2 μg/mouse] applied topically twice a week for 26 weeks. The results showed that the smoky coal sample is the most active among the three combustion emission samples. Mice were held for further observation for an additional 25 weeks after treatment. The smoky coal sample was shown to be a potent complete carcinogen, whereas the wood sample was relatively inactive as a complete carcinogen. Eighty-eight percent of the mice treated with the smoky coal sample showed carcinomas, averaging 1.1 carcinomas per tumor-bearing mouse at the end of the 77-week study. The higher tumorigenicity from the emission particles of the smoky coal combustion were in agreement with the epidemiolgical data, which showed that the Xuan Wei residents using smoky coal as a major fuel in homes had a high lung cancer mortality rate. This study demonstrates that the results of the short-term tumorigenicity assays in mice were in agreement with human lung cancer data.

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This study responds to the Agency's need to investigate the effects of indoor combustion emissions on human health in support of Clean Air Act. This work is useful in understanding the significant contribution of indoor air pollution to health effects in humans. This study indicates that indoor exposure to various types of coal and wood combustion emissions can result in different cancer risks in humans. This study provides relevant data for the EPA programs related to health effects of the products of incomplete combustion (PIC), polycyclic organic matter (POM) and wood combustion. This study also provides information on the linkage of the short-term genetic/carcinogenic assay data to epidemiology. The results are useful in the development of methods for comparative risk assessment using the short-term bioassays.

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Mouse skin tumorigenicity studies of indoor coal and wood combustion emissions from homes of residents in Xuan Wei, China with high lung cancer mortality

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The rural Xuan Wei County, Yunnan Province, China, has an unusually high lung cancer mortality rate that cannot be attributed to tobacco smoke or occupational exposure. The lung cancer rate is associated with 'smoky' coal, in contrast to wood or 'smokeless' coal burned in unventilated homes. This study was conducted to characterize and compare mouse skin tumorigenicity of the coal and the wood combustion emissions and to link the animal data to human lung cancer. Indoor air particles (< 10 μm) were collected from a central commune where the lung cancer mortality rate is high and smoky coal is the major fuel used and also from a south-western commune where lung cancer mortality rate is low and where wood or smokeless coal are the major fuels used. The organic extracts of these indoor air particles from smoky coal, smokeless coal and wood combustion were analysed for polycyclic aromatic hydrocarbons and assayed for skin tumor initiation activity and complete carcinogenicity in SENCAR mice. The results showed that the organic extract of the emission particles from smoky coal combustion is the most active in tumor initiation among the three combustion emission samples followed by smokeless coal and then wood. The organic extract of the particles from smoky coal combustion was shown to be a potent complete carcinogen, whereas the wood extract was relatively inactive as a complete carcinogen. The extract of particles from the smokeless coal combustion was not tested for complete carcinogenicity because of inadequate supply. Eighty-eight percent of the mice treated with the smoky coal extract showed carcinomas, averaging 1.1 carcinomas per tumor-bearing mouse at the end of the 77 week study. These findings were in agreement with the epidemiological data, which showed that the Xuan Wei residents using smoky coal as a major fuel in homes had a high lung cancer mortality rate. This study demonstrates that the results of the tumorigenicity assays in mice were in agreement with human lung cancer data.

Introduction

Combustion of fossil fuels and biomass generates products of incomplete combustion including polycyclic aromatic hydrocarbons (PAH*). These PAH induce tumors in experimental animals and organic extracts of particles from combustion emissions of various sources containing PAH have been shown to induce genotoxic and carcinogenic effects *in vitro* and *in vivo* (1–7). In occupational studies, coal combustion products have been associated with scrotal cancer in chimney sweeps as early as 1775 (8). Coke oven workers exposed to emissions during the carbonization of coal were found to have an increased risk of mortality from lung cancer, prostate cancer and kidney cancer (9–12). Tobacco smoke, which also contains complex mixtures of incomplete combustion products, is known to be the predominant cause of lung cancer in humans (13,14).

We investigated the relationships between lung cancer and indoor air pollution associated with coal and wood combustion in a rural county, Xuan Wei, located in Yunnan Province, China (15). Residents of Xuan Wei County, with a population of $\sim 1 \times 10^6$, have an unusually high lung cancer mortality rate that cannot be attributed to tobacco use or occupational exposure. The annual lung cancer mortality rate in Xuan Wei (26.5/100 000 during 1973 to 1979, age adjusted to the 1964 China population) is well above China's national average (5.0/100 000 during 1973 to 1975). The women in Xuan Wei have the highest lung cancer rate in China, even though the women's tobacco smoking rate is < 1%. For men in Xuan Wei, the lung cancer rate is among China's highest. The lung cancer rate varies greatly among the 20 Xuan Wei communes.

For generations, Xuan Wei residents have used one of three fuels (i.e. smoky coal, smokeless coal or wood) for heating and cooking in homes without chimneys. Lung cancer in Xuan Wei has been linked to and is highly correlated with high exposure of smoky coal combustion emission in homes, in contrast to wood or smokeless coal combustion (15). Our previous studies have shown that indoor air samples collected in the Xuan Wei communes contained mostly (51%) submicron particles with a high organic content, including high concentrations of PAH (15). The organic extracts of these samples were mutagenic in the Ames *Salmonella typhimurium*/mammalian microsome mutation assay and L5178Y $TK^{+/-}$ mouse lymphoma cell mutation assay, and these extracts transformed rodent respiratory epithelial cells *in vitro* (16). Bioassay-directed fractionation studies on the organic extracts of the smoky coal emission particles indicated the PAH and the polar fractions are the most mutagenic. Air samples from the homes in the wood-burning commune, which has a low rate of lung cancer, mostly consisted of larger particles of lower organic content and mutagenicity. Combustion of smokeless coal generates a lower level of particles than does combustion of smoky coal and these particles also contain less organics.

The mouse skin carcinogenicity assay has been a useful system for studying the multistage mechanisms of chemical carcinogenesis (17–19). The two stage model of initiation and promotion, which is operative in the mouse skin, allows for the bioassay of chemical carcinogens as well as tumor initiators and promoters (20). Complex mixtures of emissions from coke ovens, diesel and gasoline engine exhausts, roofing tar pots and cigarette smoke condensate have been examined for tumorigenicity in the mouse skin initiation/promotion assay (20–22). The carcinogenicity and tumor-initiating activity of many PAH on mouse skin also has been reported (19,23,24). Since chemical analysis

*Abbreviations: PAH, polycyclic aromatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TD_{50}, tumor initiation dose 50.

of smoky coal combustion samples has demonstrated the presence of high concentrations of PAH, that have been shown to contribute significantly to the mutagenicity of combustion products, mouse skin tumorigenicity studies were performed on the Xuan Wei combustion samples.

The objectives of this study were to (i) characterize the tumor-initiating activity and complete carcinogenicity of indoor combustion emissions (i.e. smoky coal, smokeless coal and wood) from Xuan Wei homes using the mouse skin initiation/promotion bioassay and (ii) link the mouse skin tumorigenicity data to human lung cancer in Xuan Wei.

Materials and methods

Sample collection

Inhalable particles (< 10 μm) from the combustion of smoky coal, smokeless coal and pine were collected indoors from the Xuan Wei homes during cooking periods. The samples were collected on glass fibre filters by high volume samplers with size-selective (< 10 μm) inlets as described previously (15). The smoky coal sample was collected in a central commune, Chen Guan, where the lung cancer mortality rate is high (151.8/100 000) and smoky coal is the major fuel used. Smoky coal is a low sulfur coal (0.9%) with a heating value of 27.1 MJ/kg and an ash content of 20%. This coal is comparable to US medium-volatile bituminous coal. Smokeless coal is a low-grade coal with a heating value of 14.5 MJ/kg, containing 1.9% sulfur and 49% ash. The wood and the smokeless coal samples were collected in a southwestern commune, Rhu Shui, where the lung cancer mortality rate is low (2.3/100 000). In Rhu Shui, wood represents 67% and smokeless coal represents 33% of the fuels used.

Sample preparation and PAH analysis

Organic components were removed from the filters by Soxhlet extraction using dichloromethane (Burdick and Jackson Laboratories, Inc., Muskegon, MI). The PAH content of the extracts was determined by gas chromatography/mass spectrometry as reported previously (25). The dichloromethane extracts were evaporated to a residual tar with a stream of nitrogen and were redissolved in acetone (Mallinckrodt Chemical Co., Paris, KY) for tumorigenicity assays.

Animals

Female SENCAR mice were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). Mice were housed five per cage in polycarbonate cages containing hardwood chip bedding, fed Purina Rodent Chow and water *ad libitum* and maintained under yellow light at 23.3 ± 2.2°C.

Mouse skin tumor studies

In tumor initiation studies and complete carcinogenesis studies, each treatment and control group consisted of 40 female mice, 7 to 9 weeks old. Separate positive and negative controls were conducted simultaneously with each combustion sample. All mice were shaved with electric clippers 2 days before treatment began; only mice in the resting phase of the hair growth cycle were used. Administration of all samples and controls was by dermal application of the appropriate solutions on the back with a micropipette at a volume of 0.2 ml per mouse. All samples and reagents were prepared under yellow light.

Prechronic studies. In the prechronic experiment for tumor initiation studies, groups of five mice were treated twice (on day 0 and day 7) with doses of 2, 5, 10 and 20 mg of smoky coal and wood organic extracts and 5 and 10 mg doses of the smokeless coal extract. In the prechronic experiment for the complete carcinogenesis studies, groups of five mice were treated daily for five consecutive days, rested for two days and then were treated again for five consecutive days with doses of 0.5 and 1 mg of smoky coal and wood extracts. All mice were observed for 14 days daily for toxicological and dermatologic responses and mortality.

Initiation studies. Based on the results of a 14-day range-finding prechronic study, dose levels of 1, 2, 5, 10 and 20 mg of the dichloromethane extracts of the emission particles were selected for all the samples. Due to limited sample availability of the smokeless coal sample, the 20 mg dose was not administered. All samples were administered in 0.2 ml of an acetone solution. Doses of 1, 2 and 5 mg were applied as a single topical treatment and doses of 10 and 20 mg were administered at 5 mg each on two and four successive days, respectively. One week after initiation, the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate [(TPA) CCR, Inc., Eden Prairie, MN] was topically administered, 2 μg/mouse in 0.2 ml acetone, twice weekly for 26 weeks. The control groups included (i) a positive control of benzo[*a*]pyrene-[(B[*a*]P) Aldrich Chemical Co., Milwaukee, WI] initiated (50 μg/mouse) and TPA-promoted mice and (ii) a negative control of acetone-initiated (0.2 ml/mouse) and TPA-promoted mice. All mice were observed daily for dermatologic and toxicologic responses as well as mortality. Papillomas were scored weekly according to the criteria of Bogovski (26) and papillomas were selected randomly for histologic verification. Papilloma counts vary from week to week depending on the induction, regression, or coalescing of papillomas.

Complete carcinogenesis study. Mice received the wood and the smoky coal extracts at 1 mg/mouse twice a week for 52 weeks and then were held without treatment for an additional 25 weeks. The negative and positive control groups were treated with acetone (0.2 ml/mouse) and B[*a*]P (50 μg/mouse) twice a week, respectively. All mice were observed daily for toxicologic and dermatologic responses and mortality. Tumors were counted once a week. All suspected carcinomas and atypical lesions in all mice were evaluated histopathologically according to Bogovski (26).

Data analyses and statistics

Two statistical procedures were developed to analyse mouse skin tumor data (21). The first analysis was based on tumor incidence data and used a probit model which was fitted with the use of log dose as the regression and included the TPA control tumors. The TD_{50} (the tumor initiation dose which halved the number of tumor-free mice) was estimated directly as a function of these fitted parameters and 95% confidence intervals were estimated. The second statistical model employed tumor multiplicity data and featured a nonlinear Poisson model with a background correction term. Using maximum likelihood methods, the model parameters were estimated and used to calculate the number of excess papillomas per mouse over background at a dose of 1 mg of organic extract. Asymptotic 95% confidence intervals for these activities were obtained.

Results

PAH analyses

Quantitative analyses of organic extracts from combustion emission particles were performed on selected PAH previously determined by the International Agency for Research on Cancer (6) to have sufficient evidence of carcinogenicity in experimental animals (Table I). This analysis indicated that, based on organic mass (per gram of the organic extract), high concentrations of benz[*a*]anthracene, benzofluoranthenes, benzo[*a*]pyrene, 5-methylchrysene and dibenzo[*a,e*]pyrene were found in the smoky coal sample. The smokeless coal sample showed high concentrations of benz[*a*]anthracene, benzofluoranthenes, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene and dibenzo[*a,e*]pyrene. Relative to the other samples, the wood sample contained lower quantities of these PAH. Based on air volume (per cubic metre of air), which takes into consideration the pollutant emission rate, smoky coal combustion emitted the highest concentrations for all PAH; at least one order of magnitude higher than the smokeless coal sample.

Tumor initiation

Prechronic studies with each sample indicated that SENCAR mice could support the administration of up to 20 mg of extract with no adverse dermatologic responses. In addition, no deaths, major losses in body weight or major clinical signs of toxicity were observed. Mice were initiated with the three combustion samples and then treated twice a week with TPA for 26 weeks. Beginning with the sixth week of promotion, mice were scored for papillomas weekly until the study was terminated after 26 weeks of promotion. The results of the skin tumor initiation assays of the three combustion samples and the respective positive and negative controls are presented in Figures 1–3. The survival rate was high (95% or more) for all groups of animals being studied (data not shown). Mice treated with the three combustion samples showed dose-related responses in both papilloma incidence (percentage of mice with papillomas) and tumor multiplicity (papillomas/mouse). According to the tumor incidence data, the latency period for tumor formation after initiation with the three combustion samples at the high doses (10 and 20 mg) was between 6 and 7 weeks.

The incidence of papilloma formation in mice treated with the smoky coal sample started to reach a plateau at 12–15 weeks for all doses, except the 1 mg dose (Figure 1, upper panel). The

Table I. Polycyclic aromatic hydrocarbon[a] analysis of the organic extracts of indoor air particles (PM_{10}) from different fuels burned in Xuan Wei homes

PAH	Smoky coal		Smokeless coal		Wood	
	μg/g organics	μg/m³ air	μg/g organics	μg/m³ air	μg/g organics	μg/m³ air
Benz[*a*]anthracene	1450	32.8	2060	1.01	264	4.20
5-Methylchrysene	729	16.5	464	0.23	2.0	0.03
Benzofluoranthenes	1370	31.0	3780	1.86	194	3.08
Benzo[*a*]pyrene	850	19.3	1160	0.57	204	3.24
Indeno[1,2,3-*c*,*d*]pyrene	529	11.9	915	0.45	127	2.02
Dibenzo[*a*,*e*]pyrene	771	17.5	1310	0.64	BDL[b]	BDL
Dibenz[*a*,*j*]acridine	31	0.70	14.0	0.007	BDL	BDL

[a]Compounds presented are the ones that were classified as sufficient evidence of carcinogenicity in experimental animals (6).
[b]BDL, below detection limit.

tumor multiplicity for smoky coal reached a plateau at 21 weeks in mice treated with the 10 mg dose, which was the dose level that showed the highest response among all the doses tested. The 20 mg dose of smoky coal appeared to have lower tumor initiation activity than the 10 mg dose, indicating possible toxic effects at this dose. The number of papillomas per mouse in animals treated with low doses (1, 2, 5 mg/mouse) of smoky coal continued to increase at the end of the study (Figure 1, lower panel). Other than in mice treated with the 1 mg dose, the smoky coal sample elicited papillomas in 90% or more of the treated animals at the end of the study.

At all doses, tumor incidence in mice treated with the smokeless coal sample reached a plateau before week 26 (Figure 2, upper panel). Tumor multiplicity in all groups was related directly to dose in the smokeless coal samples and continued to increase at the end of the study (Figure 2, lower panel).

In animals treated with the wood sample, the tumor incidence leveled in ~23 weeks at the two high doses (Figure 3, upper panel). Only in mice treated with the 20 mg dose was the tumor incidence >90% at the end of the study. The tumor multiplicity of the animals treated with the two highest doses of the wood sample continued to increase at the end of the study (Figure 3, lower panel). In both analyses there is an inversion in dose response about the 1 and 2 mg treatments at later weeks.

B[*a*]P-initiated mice treated with TPA for 26 weeks (positive controls) produced papilloma incidence values that ranged from 95 to 100% and papilloma multiplicity values that ranged from 5.8 to 9.0 papillomas per mouse for the three studies (data for each sample after 26 weeks of promotion shown in Figure legends). These values are well within the ranges previously reported by Nesnow *et al.* (27), except for the positive control value associated with the wood sample that gave 9.0 papillomas per mouse, a value somewhat higher than previously reported.

Complete carcinogenesis

The smoky coal and wood samples also were assayed for complete carcinogenicity at a dose of 1 mg/mouse, given twice a week for 52 weeks. The choice of the 1 mg dose was predicted on sample availability, as prechronic studies indicated no adverse dermatologic or toxicologic effects, including no losses in body weight. The mice were held for further observation for an additional 25 weeks after treatment.

After 52 weeks of treatment, 88% of the mice treated with the smoky coal sample had survived, compared with 100% of the acetone-treated mice. Mice treated with 50 μg B[*a*]P twice a week had a survival of 0% (Figure 4, upper panel). At the end of the 77 week study, the percent survival was 10, 78, and 0 for the smoky coal, acetone, and B[*a*]P controls, respectively.

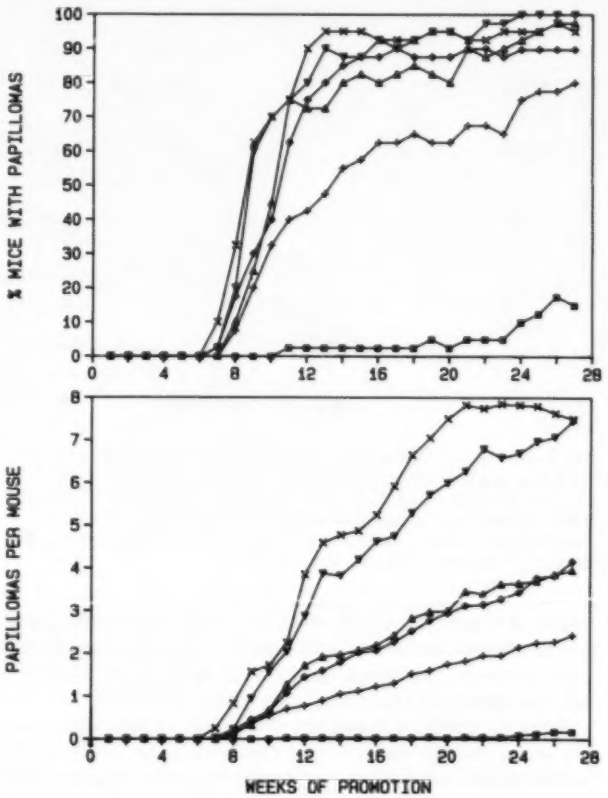

Fig. 1. Induction of papillomas in female SENCAR mice initiated with the smoky coal combustion sample and treated with TPA for 26 weeks. Mice initiated with 50 μg B[*a*]P and subsequently treated with TPA for 26 weeks exhibited a 95% papilloma incidence with an average of 7.7 papillomas per mouse. Dose of the smoky coal combustion sample: ■, 0 mg; +, 1 mg; ◆, 2 mg; ▲, 5 mg; x, 10 mg; ▼, 20 mg.

After 52 weeks of treatment, carcinomas appeared in 38% of the mice treated with the smoky coal sample (Figure 4, lower panel) with an average of 1.3 carcinomas per tumor-bearing mouse. Carcinomas began to appear at week 38. At the end of the 77 week study, 88% of the mice in this group bore at least one carcinoma, averaging 1.1 carcinomas per tumor-bearing mouse. No carcinomas were identified in the acetone control group at the end of the experiment. In the positive control animals, 55% of the mice had developed carcinomas after 29

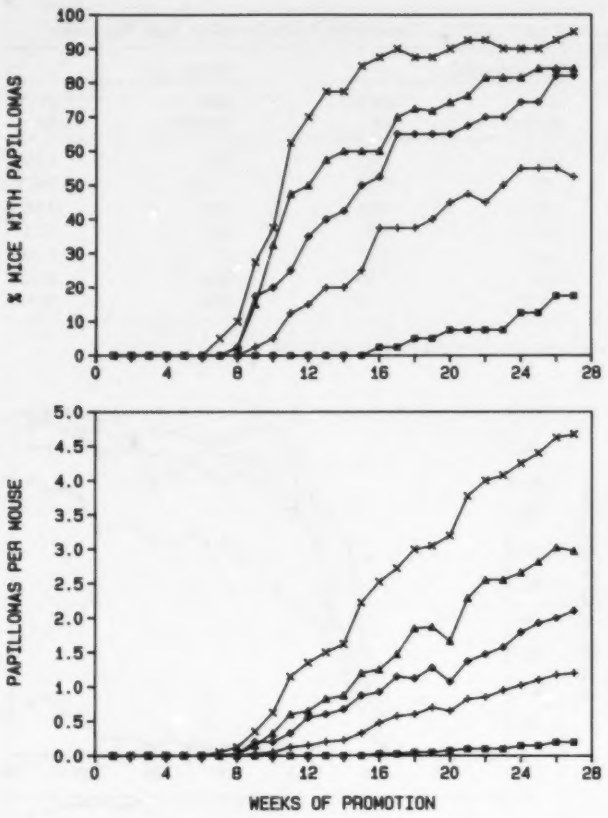

Fig. 2. Induction of papillomas in female SENCAR mice initiated with the smokeless coal combustion sample and treated with TPA for 26 weeks. Mice initiated with 50 μg B[*a*]P and subsequently treated with TPA for 26 weeks exhibited a 95% papilloma incidence with an average of 5.8 papillomas per mouse. Dose of the smokeless coal combustion sample: ■, 0 mg; +, 1 mg; ◆, 2 mg; ▲, 5 mg; x, 10 mg.

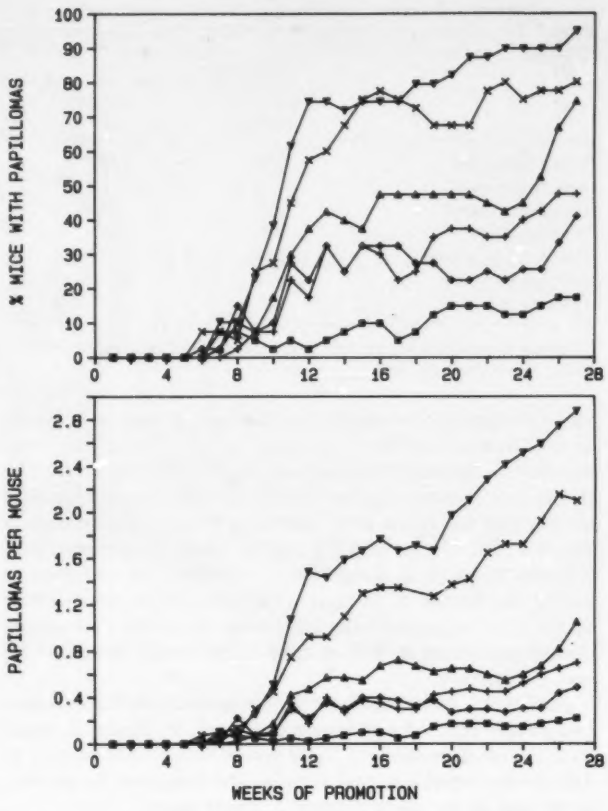

Fig. 3. Induction of papillomas in female SENCAR mice initiated with the wood combustion sample and treated with TPA for 26 weeks. Mice initiated with 50 μg B[*a*]P and subsequently treated with TPA for 26 weeks exhibited a 100% papilloma incidence with an average of 9.0 papillomas per mouse. Dose of the wood combustion sample: ■, 0 mg; +, 1 mg; ◆, 2 mg; ▲, 5 mg; x, 10 mg; ▼, 20 mg.

weeks of treatment; and by week 42 all the mice in this group had developed carcinomas, averaging 1.1 carcinomas per tumor-bearing mouse. The tumor latency for this group was 25 weeks. All the animals in this group died within 50 weeks.

After 52 weeks of treatment, 93% of the mice treated with the wood sample had survived compared with 100% of the acetone-treated mice. Mice treated with 50 μg B[*a*]P twice a week had a survival of 0% (Figure 5, upper panel). At the end of the 77 week study, the percent survival was 80, 95 and 0 for the wood, acetone, and B[*a*]P controls, respectively.

Only two mice (5% of the total animals) treated with the wood sample developed carcinomas by the end of the study, these tumors appeared at week 77. Acetone-treated mice did not develop any tumors by week 77, while B[*a*]P-treated mice exhibited a 100% incidence of carcinomas at week 44, averaging 1.5 carcinomas per tumor-bearing mouse (Figure 5, lower panel). The tumor latency for this control group was 22 weeks.

Pathological analyses

Random histopathological analyses were performed on papilloma-bearing mice from the tumor initiation studies. All mice examined were found to bear squamous cell papillomas. Histopathological analyses were performed on all mice bearing tumors after treatment with the smoky coal and wood samples and 26 of the mice treated with B[*a*]P from the complete carcinogenesis studies. Of the 34 carcinomas evaluated in the smoky coal group, 33 were found to be squamous cell in origin and one was a basal cell carcinoma. Both carcinomas found in the wood-sample-treated mice were verified as squamous cell carcinomas. All the tumors evaluated from the B[*a*]P group were squamous cell carcinomas.

Discussion

Emissions from the combustion of coal, diesel fuel, gasoline and tobacco are known to be tumorigenic in experimental animals (28–30). In the nonoccupational environment, only cigarette smoke has been unequivocally demonstrated to be carcinogenic to humans (13,14). Recently, we have reported on a population in Xuan Wei, China, with increased rates of lung cancer mortality which has been associated with the exposure to coal combustion emissions (15). In previous studies, we have shown a linear relationship between the log of the potency of emissions from coke oven, roofing tar pots and cigarettes as mouse skin tumorigens and the log of the potency of these emissions as human lung carcinogens (30). This study was conducted to characterize and compare the mouse skin tumorigenicity of the coal and the wood combustion emissions and to link the animal data to human lung cancer. The dichloromethane extracts of the indoor air particles from the typical Xuan Wei homes burning smoky coal, smokeless coal and wood were evaluated for PAH content, tumor initiation activity and complete carcinogenicity. The smoky coal

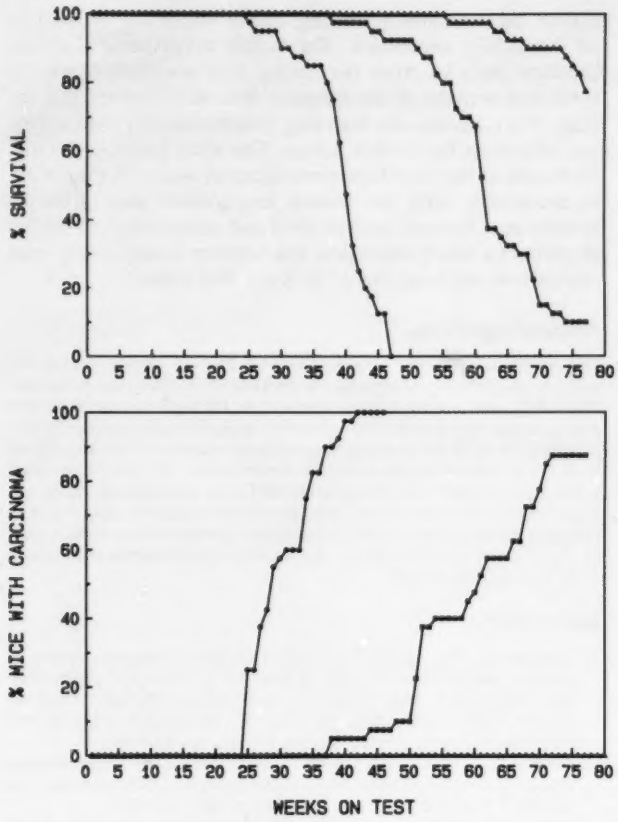

Fig. 4. Survival and carcinoma incidence in female SENCAR mice treated with the smoky coal combustion sample, B[*a*]P or acetone. Mice were treated twice weekly with the smoky coal combustion sample (1 mg/mouse), B[*a*]P (50 μg/mouse), or acetone (0.2 ml/mouse) for 52 weeks. After treatment, the mice were observed for another 25 weeks and then sacrificed. ■, smoky coal combustion sample; ●, B[*a*]P; ▲, acetone.

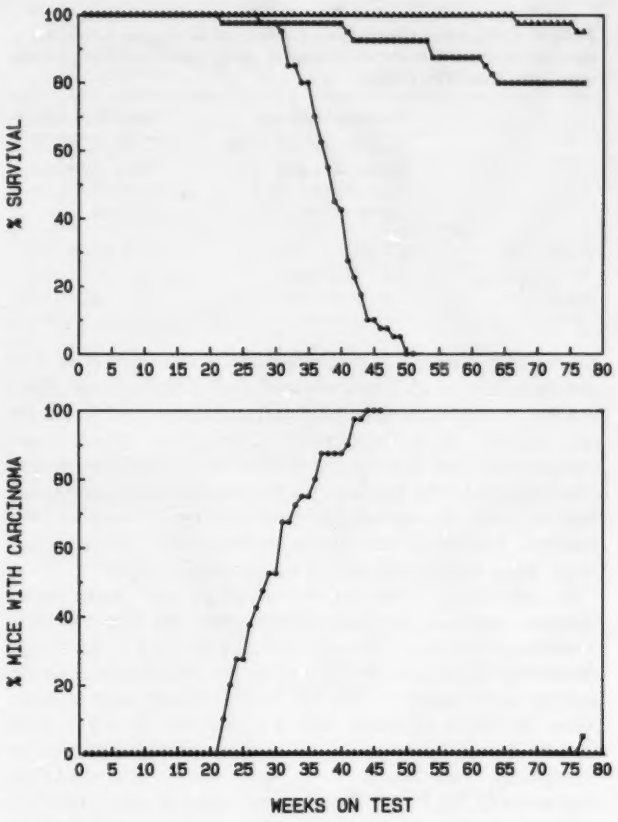

Fig. 5. Survival and carcinoma incidence in female SENCAR mice treated with the wood combustion sample, B[*a*]P or acetone. Mice were treated twice a week with the wood combustion sample (1 mg/mouse), B[*a*]P (50 μg/mouse), or acetone (0.2 ml/mouse) for 52 weeks. After treatment, the mice were observed for another 25 weeks and then sacrificed. ■, wood combustion sample; ●, B[*a*]P; ▲, acetone.

sample was collected from a home located in a commune (Chen Guan) with a high lung cancer mortality rate where smoky coal was the major fuel used. The wood and the smokeless coal samples were from the homes located in a commune (Rhu Shui) with low lung cancer rate where wood and smokeless coal were the major fuels used.

The composition and emission rates varied among the organics contained in the emission particles from burning smoky coal, smokeless coal and wood. Based on air volume (per cubic metre of air), smoky coal emitted much higher concentrations of PAH than the smokeless coal or wood. When chemically fractionated, the compositions of the coal and wood extracts also were quite different (15). More mass was distributed in the aromatic and the moderately polar fractions of the two coal extracts than in the wood extract, which contained mostly polar compounds. The organic extracts of the smoky coal and the smokeless coal were similar in the mass distribution of the organic fractions. Quantitative PAH analysis based on micrograms of PAH per gram of extract indicated that the smoky coal extract contained lower concentrations of four of the seven PAH examined compared to the smokeless coal extract, but contained a higher level of the carcinogenic nitrogen-containing heterocyclic compound, dibenz[*a*,*j*]acridine and the methylated PAH, 5-methylchrysene.

PAH are ubiquitous constituents of combustion emissions and many have been found to be carcinogenic in experimental animals (6) and the PAH fraction of combustion emissions has been shown to make a major contribution to the carcinogenic activity of those emissions (29). In these studies we found high concentrations of PAH in the organic extracts of inhalable particles from the combustion of coal. When comparing a single PAH such as B[*a*]P to the tumorigenic activity, the B[*a*]P concentrations in the two coal samples did not correspond to the tumor-initiating activity. At the 10 mg dose, the B[*a*]P concentrations of smoky coal and smokeless coal were 8.5 and 11.6 μg per mouse, respectively, while the numbers of papillomas per mouse were 7.5 and 4.7, respectively. The explanation for this may be that (i) the smoky coal contained higher concentrations of other compounds that were more potent carcinogens, such as methylated PAH or heterocyclic compounds that are known animal carcinogens and/or (ii) other compounds present in the smoky coal sample enhanced the tumor-initiating activity of B[*a*]P. For the wood sample, the wood extract contained the lowest concentration of B[*a*]P (2.0 μg per mouse) and showed the lowest tumor-initiating activity among the three samples tested (2.2 papillomas/mouse). The wood sample also showed much less activity as a complete carcinogen when compared to the smoky coal. The lack of correlation between B[*a*]P concentration and tumorigenic activity

Table II. Comparative tumor-initiating activity of the organic extracts of indoor air particles from the combustion of smoky coal, smokeless coal and wood from Xuan Wei, China

	Estimates based on nonlinear Poisson model	Estimates based on Probit model
	Papillomas/mouse at 1 mg exposure (95% confidence interval)	TD_{50}, mg/mouse (95% confidence interval)
Smoky coal	2.7 (2.5–3.0)	0.23 (0.048–1.1)
Smokeless coal	1.3 (1.1–1.5)	1.0 (0.58–1.9)
Wood	0.49 (0.39–0.63)	1.9 (1.0–3.4)

for complex mixtures is not uncommon as Grimmer *et al.* (29) and Nesnow *et al.* (21) have reported similar findings with diesel exhaust, roofing tar fumes, coke oven emissions, coal combustion and cigarette smoke condensate. Combustion emissions are complex mixtures that contain classes of chemical carcinogens other than PAH. Furthermore, such mixtures contain agents that may enhance the tumorigenic activities of carcinogens. For example, pyrene, a cocarcinogen in mouse skin (31), is present in all three combustion emissions investigated here.

A comparison of the tumor multiplicity data in the tumor initiation assay for the three samples, after the data was fit to a nonlinear Poisson model, indicated that the smoky coal sample showed the highest activity followed by the smokeless coal sample and the wood sample (Table II). Similar results were obtained when the tumor incidence data was analysed by a log-probit model. The relative activities of the three samples based on the multiplicity data (wood = 1) were smoky coal:smokeless coal:wood (5.5:2.7:1.0). Based on the tumor incidence data, the relative activities of the three samples were 8.3:1.9:1.0, respectively. This suggests that the smoky coal sample is more active than the smokeless coal, which is more active than the wood sample, based on the amount of organic material applied to the mice. As a complete carcinogen, the smoky coal sample was quite potent in inducing squamous cell carcinomas after a one-year treatment and a 25-week additional period of observation, while the wood sample was relatively inactive.

The magnitude of human cancer risk from exposure to combustion emissions depends on duration and frequency of the exposure, the types of the compounds contained in the mixture of the combustion emission, and concentrations of the active carcinogenic compounds present in the combustion emission. In order to use the mouse skin tumor data to understand the potential hazards related to exposure to emissions from smoky coal, smokeless coal and wood, a comparison needs to be made based on air volume (micrograms of organics per cubic metre of air). Previously reported organic emissions for the three fuels were smoky coal, 17.6 mg/m^3; smokeless coal, 0.5 mg/m^3; and wood, 12.3 mg/m^3 (32). Based on this air monitoring data and the tumor data presented here, residents in Chen Guan that burn smoky coal are exposed to much higher concentrations of a more tumorigenic organic material than are the residents in Rhu Shui that use wood and smokeless coal. These differences also need to be interpreted in light of the fact that the organic extracts tested in the mouse skin studies are derived from combustion emission particles and do not contain all of the toxicants (e.g. volatiles, metals) to which the human population is exposed.

In summary, Xuan Wei residents burning coal and wood in unvented homes were exposed to different concentrations of organic pollutants which showed differences in tumor-initiating activity and carcinogenicity in mouse skin. These findings may explain the variations in the lung cancer rate of the residents in the Xuan Wei communes. The higher tumorigenicity of the emission particles from the smoky coal combustion was in agreement with the epidemiological data which showed that the Xuan Wei residents with high lung cancer mortality used smoky coal as a major fuel in their homes. This study demonstrates that the results of the short-term tumorigenicity assays in mice were in accordance with the human lung cancer data. The air monitoring, chemical, toxicological and epidemiological studies all point to a strong etiological link between indoor smoky coal combustion and lung cancer in Xuan Wei county.

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